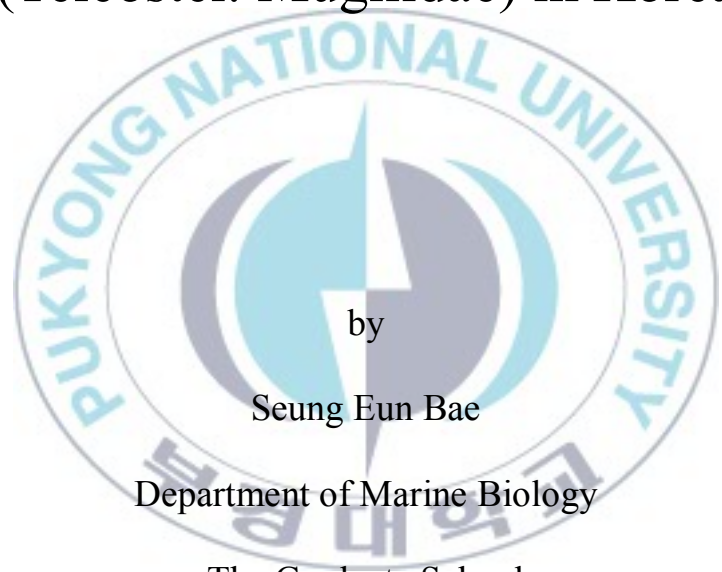
Thesis for the Degree of Master of Science

Morphological and genetic variation of geographic populations of the flathead mullet, *Mugil cephalus* (Teleostei: Mugilidae) in Korea



by

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The Graduate School

Pukyong National University

August 2014

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[한국산 숭어 (숭어목: 숭어과) 지역 집단 간 형태 및 유전변이]

Advisor: Prof. Jin Koo Kim

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

in Department of Marine Biology, The Graduate School,

Pukyong National University

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August 21, 2014

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한국산 숭어 (*Mugil cephalus*) 지역 집단 간 형태 및 유전변이

배승은

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요약

숭어과 어류는 형태적으로 매우 유사하여 분류학적으로 논란이 많은 그룹으로, 현재까지도 이들의 명명법과 계통관계는 불분명하다. 이러한 이유로 숭어과 어류는 233종의 nominal species가 보고되어 있지만, 이 중 80여종만이 현재 valid species로 인정받고 있다. 최근에는 분자분석을 이용하여 숭어과 어류를 동정하거나 계통관계를 밝히고자 하는 연구가 많이 이루어지고 있다. 특히, 숭어는 전세계적으로 지리적 분포에따른 유전적 변이로 최소 14개의 소그룹으로 나누어진다. 본 연구는 우리나라에 서식하고 있는 숭어집단 (고성, GS; 부산, BS; 여수, YS; 완도, WD; 부안. BA; 제주도, JJ)을 대상으로 형태와 mtDNA COI 및 16s rRNA 염기서열을 비교하여 숭어의 계통학적 위치를 밝히고자 하였다.

형태적으로 제주도 개체군은 다른 지역 개체군보다 두장, 체고, 미병고에서 평균적으로 더 높은 값을 나타내었다. CDA 분석결과에서는 총 5개의 정준판별함수가 산출되었고, 제 1판별함수가 73.0%로 기여도가 가장높았다. 제 1판별함수는 미병고에서 가장 큰 절대값을 나타내었고 (-2.753), 산점도에서도 제주도 지역 집단이 다른 지역 집

단과 확연히 분리되었다. MtDNA COI 572bp와 16s rRNA 541bp를 증폭시킨 결과, 크게 두 개의 그룹으로 나누어졌다. 첫 번째 그룹에는 모든 지역 개체군이 포함되었으나, 두 번째 그룹에는 JJ 개체군과 SH개체군만이 포함되었다. Bayesian 분석결과도 NJ 분석결과와 마찬가지로 2개의 그룹으로 나누어졌다. 두 그룹 사이의 유전적 거리는 COI에서는 d = 0.021–0.029, 16s rRNA에서는 d = 0.008–0.012로 나타났다. 대만의 숭어 3그룹의 염기서열과 비교한 결과, 첫 번째 그룹은 lineage 1에, 두 번째 그룹은 lineage 2에 포함되었다.

두 그룹은 약 1~1.4MY 전에 분화한 것으로 추정되며, 빙하기 동안 해수면의 하강에 따른 지리적 고립이 발생한 것으로 생각된다. 또한, 우리나라 주변에는 대마난류, 중국대륙연안수, 남해연안수 등 다양한 수괴가 출현하며, 이러한 해류의 흐름은 숭어의 분포와 이동을 제한하는 장벽 역할을 하는 것으로 추정된다.

결론적으로, 형태와 분자분석을 근거로 우리나라 숭어는 2개의 lineage로 나누어지며, 특히 제주도 지역 개체군은 두 개의 lineage가 공존하는 것으로 나타났다. 두 집단이 별종인지에 대한 여부는 추후 microsatellite분석과 골격적 차이를 비교한 후 종전까지 숭어의 동종이명으로 보고된 종들과의 종합적인 비교, 검토가 필요할 것으로 사료된다.

I. Introduction

Family Mugilidae comprises 72 species in 17 genera worldwide (Nelson, 2006), and atotal of 7 species in 5 genera including recently reported in Korea (Kim et al., 2005; Kwun et al., 2013): *Mugil cephalus* Linnaeus, 1758, *Chelon haematocheilus* (Temmink and Schlegel, 1845), *Chelon affinis* (Günther, 1861), *Chelon macrolepis* (Smith, 1846), *Moolgarda seheli* (Forsskål, 1775), *Oedalechilus labiosus* (Valenciennes, 1836), *Ellochelon vaigiensis* (Quoy and Gaimard, 1825). Among them, genus *Mugil* includes only one species, *Mugil cephalus*.

Mugil cepahlus are globally distributed in tropical, subtropical and temperate coastal waters of all seas, and this species migrate to the sea to spawn (Ke et al., 2009; Whitfield et al., 2012). Despite ecologically important species, the taxonomic and evolutionary relationship of *Mugil cephalus* remained unclear (Harrison et al., 2007; Durand et al., 2012). The major reason is that because Mugilidae fishes have conservative morphology, there are few morphological character to reveal their systematics (Semina et al., 2007; Heras et al., 2009).

Many authors tried to identify the taxonomic status of mugilid fishes using

major morphometric characters such as the shape of maxilla, the shape and the number of scales, but the results were mostly contentious and failed to prove the identity of species of mugilidae. So, mugilids are known as one of the most difficult taxonomic groups (Durand et al., 2012; Ashiq Ur Rahman et al., 2013; Kwun et al., 2013). Schultz (1946) mainly used mouth anatomy and reproductivity in order to define both mugilidae genera and species, and Thomson (1997) tried to establish phylogenetic relationships within the mugilidaebased on both internal (intestine, stomach, pyloric caeca) and external (nostrils, teeth, scales, lips, jaw) anatomical structures.

In Korea, there have been some studies to compare between species or within species using morphological characters such as pyloric caeca, lateral line scales, the shape of maxilla and upper jaw teeth produced by Lee and Joo (1994), and Kim and Kim (1998). Also, Kim (1999) compared three mugilid fishes in Korea based on external morphology, skeleton structres, early life history and genetic relationships to provide the evidence of monophyly of the family mugilidae.

These cryptic species are indistinguishable due to have very similar morphological character, and there is a limit to find the suitable character for distinct species identification. So, recently, DNA molecular analysis are used to

identify mugilid fishes (Kwun et al., 2012b; Kwun et al., 2013) or reveal the phylogenetic relationships within mugild fishes (Kim et al., 2003; Ke et al., 2009; Liu et al., 2009a, 2009b; Durand et al., 2012; Durand et al,. 2013).

Durand et al. (2012) proposed when compared phylogenetic relationships within mugilidae species in the world using three mtDNA loci (COI, 16s rRNA, Cyt b), globally-distributed *Mugil cephalus* comprises at least 14 different groups. However, although *Mugil cephalus* was divided into several groups, the *Mugil* species clustered into a single, well-supported clade, and this genetic differentiation was regarded as intraspecific level (Durand et al., 2012). Also, Ke et al. (2009) showed that three lineages of *Mugil cephalus* existed sympatrically in Taiwan on the basis of molecular analysis in cytochrome b. According to Liu et al. (2009) and Sun et al. (2012), there are two groups in China, supported by analysis of mtDNA control region and COI. And Shen et al. (2012) suggested that *Mugil cephalus* in northwestern pacific was divided into three lineages, and among them, *Mugil cephalus* in East China Sea including Korea comprises only one groups.

In short, globally-distributed *Mugil cephalus* showed regionally genetic differentiation, and it is revealed that there are two or three groups of *Mugil cephalus*, distributed in Taiwan and China around Korea, by the molecular

analysis. So, the hypothesis in this study is as follows: *Mugil cephalus* populations around Korea will be genetically different from each other, and be divided into two or three groups through molecular analysis.

The purpose of this study is to test whether there is genetic differentiation of *Mugil cephalus* around Korea, and to reveal phylogenetic relationships of *Mugil cephalus* by morphological and molecular analysis using mtDNA COI and 16s rRNA genes.

II. Materials and Methods

1. Sample collection

Specimens were collected from six locations along the Korean coasts between 2008 and 2014 (Fig. 1), and the number of speciemens in each sites were as follows: Goseong (n=29), Busan (n=29), Yeosu (n=26), Wando (n=31), Buan (n=31), Jeju Island (n=42). Speciemens were preserved in 70% ethoanol after fixed in 15% formaldehyde for a week. The samples in this studyhave been deposited at the ichthyology lab in Pukyong National University (PKU), Korea.

2. Morphological analysis

In order to compare morphological differences among populations in each sites, 7 counts and 21 measurements were analyzed. Seven meristic characters included the number of first dorsal fin spine, second dorsal fin rays, pectoral fin rays, pelvic fin spine and rays, and measurements were a total of 21 characters

including interorbital width and body depth (Fig. 2). Counts and measurements followed Nakabo (2002) using a vernier caliper to the nearest 0.1mm. Statistical analysis in measurements was performed using canonical discriminant analysis (CDA). This analysis was carried out in SPSS 12.0 software for Windows and applied the proportion with standard length on 19 measurements.

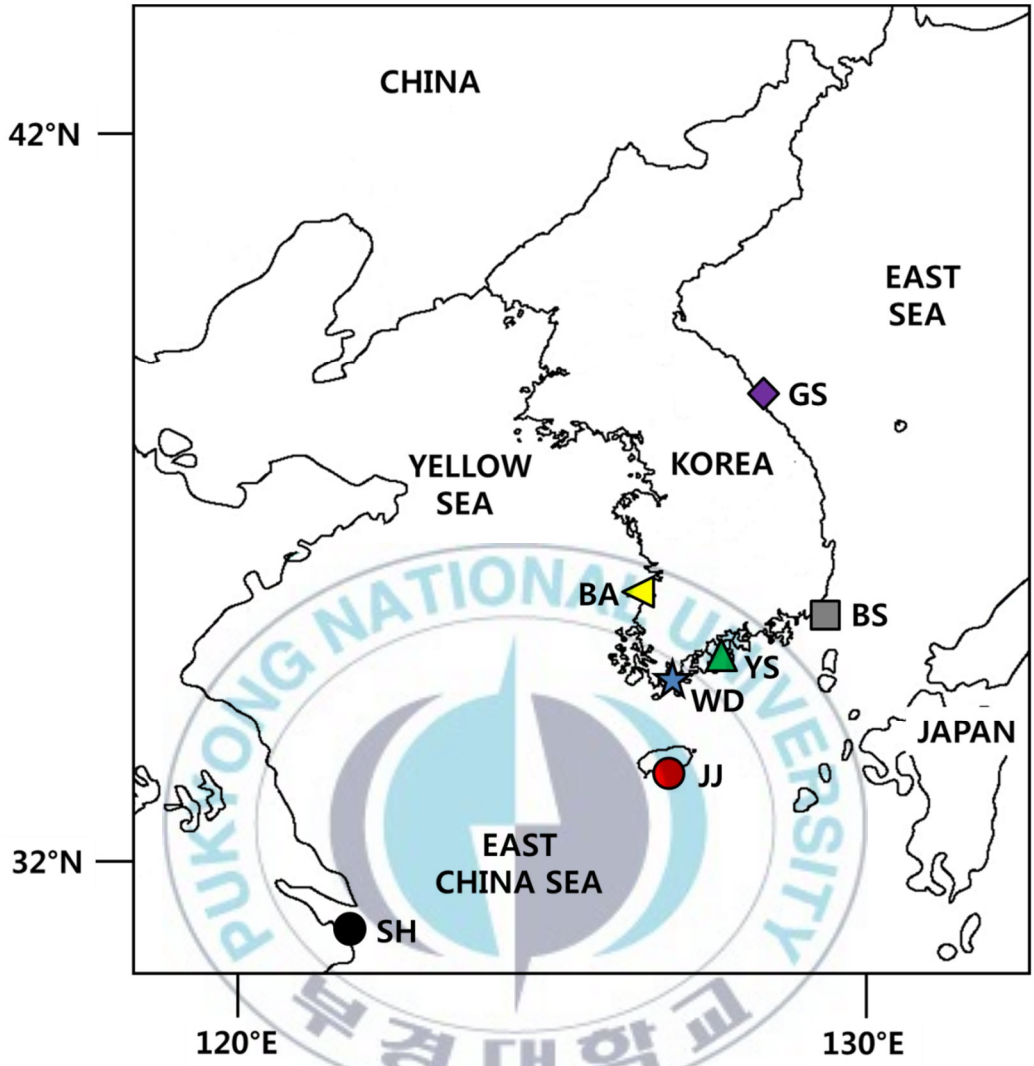

Fig. 1. Sampling sites of *Mugil cephalus*: GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

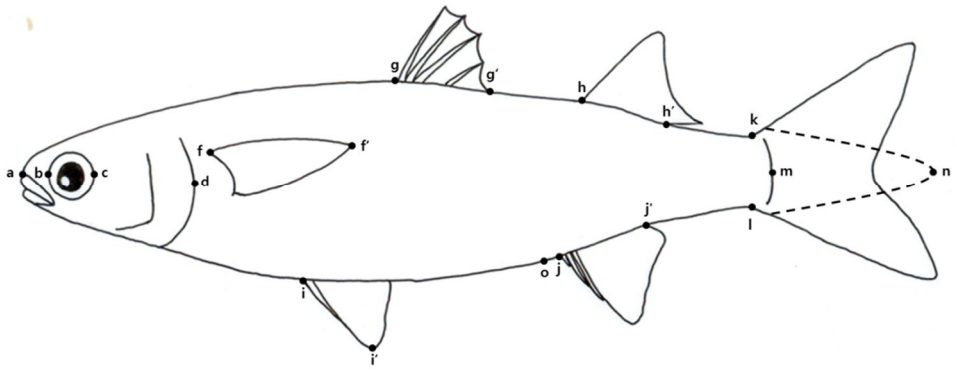

Fig. 2. The measuremetnsof *Mugil cephalus*. a~n: Total length (TL); a~m: Standard length (SL); a~d: Head length (HL); a~b: snout length; b~c: orbital length; c~d: posterorbital length; a~g: pre-1st dorsal fin length; a~h: pre-2nd dorsal fin length; a~f: pre-pectoral fin length; a~i: pre-pelvic fin length; a~o: pre-anus length; a~j: pre-anal fin length; g~g': 1st dorsal fin base length; h~h': 2nd dorsal fin base length; f~f': pectoral fin length; i~i': pelvic fin length; j~j': anal fin base length; j'~m: caudal peduncle length; k~l: caudal peduncle depth.

3. Molecular analysis

i. DNA extraction and PCR sequencing

Total DNA was extracted from muscle tissue using Chelex 100 resin (Bio-rad, USA). Eight specimens from shanghai (SH) were used asgenetic comparing group because having only tissue in pectoral fin instead of fish body. MtDNA cytochrome oxidase subunit I (COI) and 16s ribosomal RNA were amplified to compare the genetic differentiation among populations in each site. The COI and 16s rRNA of mitochondrial DNA by PCR with universal primers, and each primer set was used for amplification: mtDNA COI primer set with VF2 (5'–TCAACCA ACCACAAAGACATTGGCAC–3') and FishR1 (5'–TACACTTCTGGGTGGCC AAAGAATCA–3'), and 16s rRNA primer set with 16S ARL (5'–CCGGTCTAGA CTCAGATCACGT–3') and 16S BRH (5'–CGCCTGTTTATCAAAAACAT–3') (Ivanova et al., 2007). The PCR solution contained 2μL of genomic DNA, 3 μL of 10x buffer, 2.4 μL of dNTPs, 1 μL of each primer, 0.1 μL of Taq polymerase (Biomedic, Korea), and distilled water to bring the final volume to 30 μL. The PCR was performed under the following conditions for each gene: In COI sequnces, initial denaturation was for 1 min at 95 ℃, followed by 35 cycles of 1

min at 95℃ for denaturation, 1 min at 53℃ for annealing, and 1 min at 72 ℃ for extension, with a final extension at 72 ℃ for 5 min. In 16s rRNA sequences, thermal profile began at 95 ℃ for 11 min, followed by 35 cycles of 94℃ (1 min), 53℃ (1 min), and 72℃ (1 min), with a final step of 5 min at 72℃.

ii. Sequence alignment and phylogenetic analysis

After PCR sequencing, mixture with 5 ㎕of PCR products and 2㎕ of 6X loading buffer was injected into 1% agrose gel, and DNA electrophoresis was performed on 100 voltage for 25 minutes. And then, amplified products were checked after dyed using Noble view nucleic acid stain solution. The PCR products were purified with Davinch^{TM} PCR Purification Kit (Davinch-K, Seoul, Korea), anddirectly sequenced with the ABI Bigdye terminator cycle sequencing ready reaction kit v.3.1 (Applied Biosystems Inc., USA)and run onABI 3730XL sequencer (Applied Biosystems Inc., USA).

The mtDNA COI and 16s rRNAsequenceswere aligned using ClustalW (Thompson et al., 1994) in BioEdit version 7 (Hall, 1999), and genetic distances

were calculated using the Kimura-2-parameter model (Kimura, 1980) in MEGA 5 (Tamura et al., 2011). The phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 5and its confidence was assessed via 1,000 bootstrap replications (Tamura et al., 2011). Also, the best-fit model test of each sequences evolution were selected using MrModeltest v.2.3 (Nylander, 2004). The selected model were both HKY+I model for COI and 16s rRNA. The phylogenetic trees were construced using BEAST 1.7.5 (Drummond and Rambaut, 2007), and importing taxa and specifiying the evolutionary models were done in BEAUti (Drummond et al., 2012). The Markov chain Monte Carlo (MCMC) analyses of each region were run for 10 milion generations, and the consensus trees along with posterior probabilities were visualized using FigTree Ver.1.4.0 (Rambaut, 2012).

The sequences of three lineages in Taiwan, suggested by Ke et al. (2009), were used to confirm the lineages of *Mugil cephalus* in Korea: COI-JQ060540, JQ060541, JQ060553; 16s rRNA-JQ060778, JQ060789, JQ060801. And *Chelon haematocheilus* (PKU 2544) was used as an outgroup.

iii. Data analysis

The molecular diversity indices for each region, such as haplotype diversity and nucleotide diversity, analysis of molecular variance (AMOVA), pairwise *F*st values, and Tajima's D and Fu's Fs test of neutrality were calculated in Arlequin 3.5.1.2. Historic demographic expansion were analyzed using mismatch distribution analysis in Arlequin v.3.5.1.2, and Minimum Spanning Network (MSN) were constructed using TCS v.1.21 program.

III. Results

1. Morphological analysis

The results of meristics and measurements were shown Table 1 and Table 2. Compared to 7 counts characters, six populations (GS, BS, YS, WD, BA, JJ) were similar in most of meristic characters, but juveniles in JJ population have different from adults based on the number of anal fin spines and rays (II, 9 in juvenile vs. III, 8 in adult) (Table 1). For the morphometric measurements, the average of the body depth and caudal fin depth were higher in JJ population. Also, juveniles in JJ population have higher value for the head length, eye diameter, pre-1st dorsal fin length, pre-pectoral fin length, pre-pelvic fin length, pectoral fin length, pelvic fin length, and anal fin base length than others (Table 2).

In order to determine which morphometric measurement most effectively differentiates populations, canonical discriminant analysis (CDA) was examined. As the results, a total of five canonical variables were suggested (Table 3). The first canonical variables (CAN 1) contributed 73.0% of the total variation (the eigenvalue of CAN 1 was 4.536), and the second canonical variables (CAN 2) accounted in 12.4% of the total variation (the eigenvalue of CAN 2 was 0.772).

The characters of primary importance in distinguishing between the groups were the caudal peduncle depth for the first canonical variable (-2.753), and the head length for the second variable (-12.419). The results of expected affiliated groups showed that six populations could be classified correctly with an accuracy of 73.9%. Among them, JJ population has the highest classification criterion (85.7%), but the lowest in WD population (58.1%). The plot of CDA showed that five populations (GS, BS, YS, WD, BA) were overlapping in both axes, but JJ population was morphologically distinct from others although four specimens overlapped with others (Fig. 3). Therefore, JJ population was divided into other populations on the basis of the caudal peduncle depth.

Table 1. Frequency of occurrence of dorsal fin spines and rays, anal fin spines and rays, and pectoral fin rays of *Mugil cephalus* from Korea.

	Dorsal fin spines	Dorsal fin rays		Anal fin spines		Anal fin rays		Pectoral fin rays					
Region	IV	8	9	II	III	8	9	14	15	16	17	18	19
GW	29		29		29	29			1	12	13	2	1
BS	29		29		29	26	3		8	12	7	2	
YS	26		26		26	26			2	11	11	2	
WD	31		31		31	31		1		12	15	3	
BA	31		31		31	31			2	9	16	4	
JJ (adult)	15		15		15	15			3	4	5	2	1
JJ (Juvenile)	27	1	26	27			27		1	6	10	9	1

GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

Table 2. Comparison of meristics and measurements of *Mugil cephalus* in

	Adult				
	GS	**BS**	**YS**	**WD**	**BA**
Number of specimens	29	29	26	31	31
Total length (mm)	242.00-543.00	154.00-553.00	324.00-470.00	140.00-604.00	412.00-538.0
Standard length (mm)	195.52-440.00	124.74-444.00	260.00-379.00	111.63-500.00	326.00-443.0
% In standard length					
Head length	24.04-26.49 (25.30)	24.36-27.80 (26.07)	24.65-27.16 (25.90)	24.28-27.62 (25.46)	23.77-27.18 (25.01)
Orbital length	4.81-6.05 (5.51)	5.06-7.19 (6.12)	4.50-6.13 (5.43)	4.25-7.21 (5.18)	4.63-5.83 (5.2
Snout length	6.00-7.59 (6.98)	6.16-7.60 (6.82)	6.58-7.90 (7.28)	5.90-8.04 (7.21)	6.20-7.78 (6.9
Post-orbital length	13.21-14.88 (14.01)	12.83-15.53 (14.37)	12.97-15.08 (14.35)	13.24-15.07 (14.31)	12.67-15.81 (14.08)
Interorbital width	10.49-13.38 (12.25)	10.70-13.52 (11.90)	11.42-14.57 (12.55)	10.07-13.86 (12.35)	10.66-12.78 (11.47)
Body depth	18.86-23.47 (20.76)	17.64-24.76 (21.43)	16.54-22.67 (19.74)	17.45-20.84 (19.09)	17.43-22.22 (19.65)
Pre-1st dorsal fin length	21.80-50.10 (47.51)	46.46-49.95 (48.33)	46.95-49.50 (48.42)	46.53-51.10 (48.25)	45.94-49.02 (47.66)
Pre-2nd dorsal fin length	71.08-74.73 (72.85)	70.84-74.75 (72.56)	71.22-74.76 (72.87)	70.42-74.41 (72.33)	69.88-74.30 (71.75)
Pre-pectoral fin length	25.43-49.74 (28.29)	25.97-30.53 (27.60)	25.80-28.20 (27.08)	25.17-30.09 (26.76)	25.30-27.91 (26.41)
Pre-pelvic fin length	36.74-39.30 (38.01)	37.44-41.94 (39.01)	36.89-40.57 (38.37)	36.78-39.47 (38.04)	36.67-41.94 (38.10)
Pre-anus length	66.07-70.99 (68.40)	66.90-70.77 (68.67)	65.11-71.88 (68.15)	65.80-73.09 (68.11)	63.52-70.55 (68.60)
Pre-anal fin length	69.55-73.53 (71.42)	69.64-73.17 (71.40)	67.90-73.36 (70.74)	68.04-75.58 (71.20)	70.39-74.63 (72.31)
1st dorsal spine length	6.99-13.19 (11.32)	7.16-12.96 (10.00)	7.54-14.17 (12.10)	7.41-12.88 (11.07)	9.69-13.27 (11.
2nd dorsal fin base length	10.71-12.19 (11.65)	10.69-12.96 (11.82)	10.96-13.12 (12.07)	11.03-12.50 (11.82)	10.77-12.67 (11.76)
Pectoral fin length	16.52-18.49 (17.43)	15.70-18.97 (17.60)	13.30-18.55 (16.90)	11.57-18.04 (15.38)	14.21-18.38 (16.91)
Pelvic fin length	13.06-15.82 (14.71)	13.20-17.00 (15.21)	12.54-16.65 (14.88)	11.95-15.93 (13.89)	12.81-15.37 (14.28)
Anal fin base length	10.87-12.96 (11.93)	11.26-13.88 (12.32)	11.31-18.77 (12.52)	10.63-12.81 (11.87)	10.87-12.79 (11.92)
Caudal peduncle length	17.19-19.40 (18.66)	17.47-20.83 (19.28)	17.96-20.35 (19.01)	17.62-20.01 (18.91)	18.28-20.26 (19.12)
Caudal peduncle depth	8.62-9.62 (9.13)	7.98-9.64 (8.99)	7.98-9.70 (9.10)	8.13-9.81 (9.00)	8.48-9.56 (9.0

Table 3. Standardized canonical (CAN) coefficients based on 19 morphometric characters of *Mugil cephalus* in 6 populations.

Measurements	CAN1	CAN2	CAN3	CAN4	CAN5
Head length	1.488	-12.419	0.256	-7.731	-0.548
Orbital length	0.146	3.977	1.564	1.400	0.375
Snout length	0.084	0.454	-0.726	0.208	-0.107
Post-orbital length	1.079	5.577	-3.518	6.058	-1.564
Interorbital width	0.069	1.251	-1.433	-0.406	-0.141
Body depth	-0.933	-0.650	1.448	-0.804	0.835
Pre-1st dorsal fin length	-0.062	-1.007	-1.147	1.499	-1.051
Pre-2nd dorsal fin length	-1.802	9.348	-7.927	-8.857	5.241
Pre-pectoral fin length	0.329	0.389	2.145	-2.469	2.279
Pre-pelvic fin length	0.533	-0.459	0.779	4.194	-0.760
Pre-anus length	-1.217	-1.606	3.365	-1.205	1.433
Pre-anal fin length	1.446	-3.168	3.367	3.575	1.191
1st dorsal spine length	0.171	-0.509	0.645	-1.597	-1.944
2nd dorsal fin base length	1.109	-2.347	-0.484	1.409	0.641
Pectoral fin length	-2.122	2.521	3.704	2.187	-1.700
Pelvic fin length	1.738	1.508	-0.991	-3.095	-2.174
Anal fin base length	0.868	-0.514	1.643	-0.994	-2.020
Caudal peduncle length	0.671	-0.323	-3.072	3.392	-2.749
Caudal peduncle depth	-2.753	-1.789	0.592	3.279	2.795
Eigenvalues	4.536	0.772	0.627	0.160	0.117
Proportions (%)	73.0	12.4	10.1	2.6	1.9
Cumulative values (%)	73.0	85.5	95.5	98.1	100.0

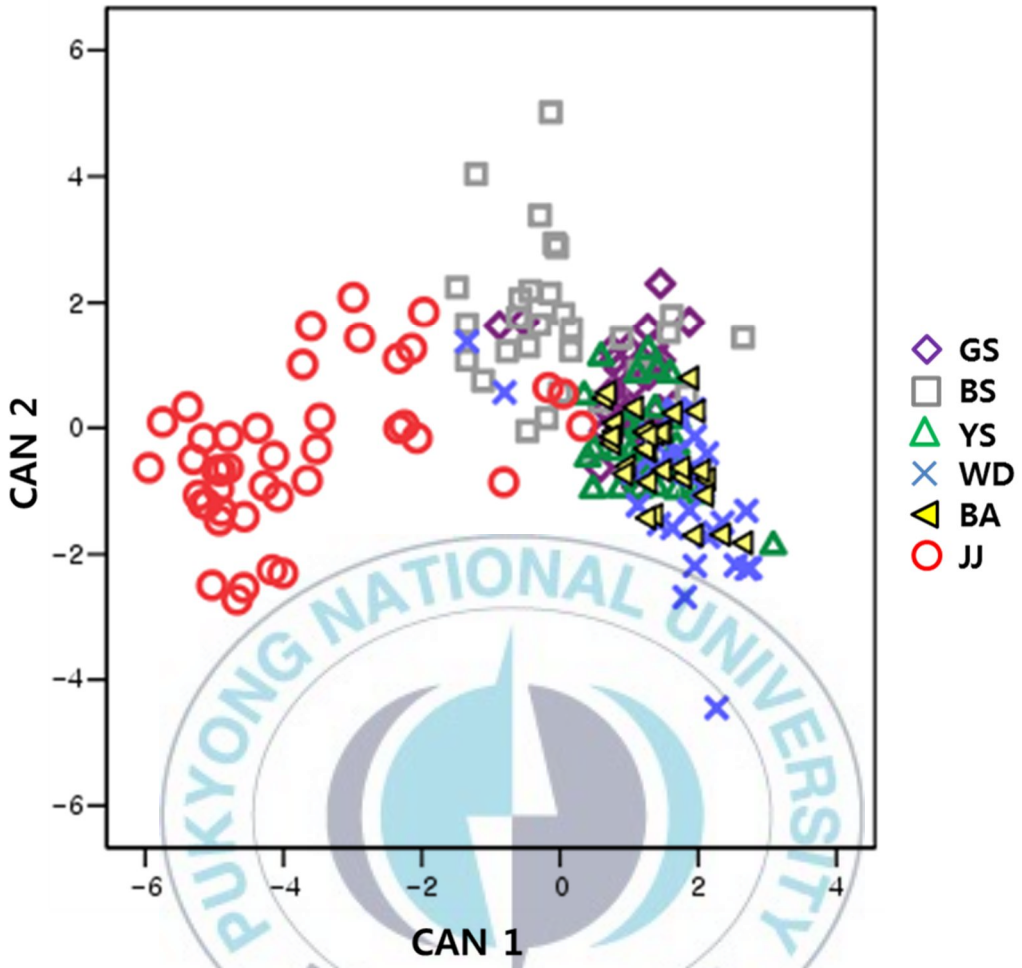

Fig. 3. The plots of canonical discriminant scores the first and second canonical (CAN) axes among *Mugil cephalus* based on 19 morphometirc characters. GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

2. Molecular analysis

i. Genetic diversity

MtDNA COI 572 base pair (bp) was amplifiedin order to compare GS (n=29), BS (n=29), YS (n=26), WD (n=31), BA (n=31), JJ (n=42), and SH (n=8). As the results, a total of 44 polymorphic sites was detected, with 36 transitions, 8transversions, and 44 substitutions. Also, a total of 18 haplotypes were found, andamong them, haplotype 1 (H1) and haplotype 12 (H12) were the most dominant (Table 4). H1 was shared by all populations and H12 was shared by only JJ population. Haplotype diversity is the highest in JJ population, but the lowest in BA populations. Also, nucleotide diversity showed that JJ population is the highest, but the lowest in BS populations (Table 5). When compared to pairwise Fst between JJ population and other populations, the range was from 0.58633 to 0.76325 and this indicates significant differentiation among populations ($p < 0.001$) (Table 6).

When amplified 541bp 16s rRNAfragment, a total of 28 polymorphic sites was detected, with 27 transitions, 1 transversions, and 28 substitutions. Also,a total of 15 haplotypes were found, and among them, H1 and H12 were the most frequent haplotypes (Table 7). Similar to COI results, all populations possessed

H1, and only JJ population shared H12. In the haplotype diversity, JJ population is the highest, but the lowest in YS population. And, JJ population has the highest nucleotide diversity, but the lowest in GS population (Table 8). Compared to pairwise Fst values, JJ population showed significant differentiation from others, ranging from 0.60404 to 0.77294 ($p < 0.001$) (Table 9). This indicated a clear differentiation between JJ population and others.

Table 4. Distribution of mtDNA COI haplotypes in six populations of *Mugil cephalus*.

Haplotype	Sampling site							n	%
	GS	BS	YS	WD	BA	JJ	SH		
H1	26	27	23	26	29	7	6	144	73.47
H2	2							6	3.06
H3	1							1	0.51
H4		1						1	0.51
H5		1						1	0.51
H6			1					1	0.51
H7			1					1	0.51
H8			1					1	0.51
H9				1				1	0.51
H10					1			1	0.51
H11					1			1	0.51
H12						21		21	10.71
H13						9	1	10	5.10
H14						2		2	1.02
H15						1		1	0.51
H16						1		1	0.51
H17						1		1	0.51
H18							1	1	0.51
Total	29	29	26	31	31	42	8	189	100

GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

Table 5. Summary of molecular diversity for *Mugil cephalus* in mtDNA COI. Number of individuals (n), number of haplotype (*N*), haplotype diversity (*h*), nucleotide diversity (π), mean number of pairwise differences (*k*) for each population of samples. Tajima's D and Fu's Fs, corresponding P value, and mismatch distribution parameter estimates for each population were also indicated.

						Tajima's *D*		Fu's *Fs*		Mismatch distribution		
Region	**n**	***N***	***h***	**π**	***k***	***D***	***P***	***Fs***	***P***	**τ**	**θ0**	**θ1**
Goseong (GS)	29	3	0.1970 ± 0.0952	0.0004 ± 0.0005	0.2020 ± 0.2571	-1.249	0.082	-1.628	0.021	3.000	0.000	0.260
Busan (BS)	29	3	0.1355 ± 0.0845	0.0002 ± 0.0004	0.1379 ± 0.2084	-1.509	0.015	-2.312	0.007	3.000	0.000	0.164
Yeosu (YS)	26	4	0.2215 ± 0.1063	0.0004 ± 0.0005	0.2308 ± 0.2780	-1.734	0.008	-3.147	0.001	3.000	0.000	0.299
Wando (WD)	31	3	0.2882 ± 0.0971	0.0005 ± 0.0006	0.2968 ± 0.3196	-0.826	0.199	-0.899	0.190	3.000	0.000	0.425
Buan (BA)	31	3	0.1269 ± 0.0798	0.0003 ± 0.0005	0.1935 ±0.2506	-1.731	0.013	-1.668	0.029	3.000	0.000	0.122
Jeju Island (JJ)	35	7	0.6887 ± 0.0570	0.0086 ± 0.0048	4.9408 ± 2.4537	0.587	0.767	3.970	0.925	0.000	0.000	99999.0
Shanghai (SH)	8	3	0.4643 ± 0.2000	0.0061 ± 0.0040	3.5000 ± 1.9914	-1.791	0.004	2.952	0.913	0.027	0.000	99999.0

Table 6. Pairwise estimates of *F*st (below the diagonal) and Pairwise *F*st *P* values populations of *Mugi l cephalus* in mtDNA COI.

Locality	GS	YS	WD	BA	J
Goseong (GS)		0.31532	0.58559	0.19820	0
Busan (BS)	0.01429	0.47748	0.09910	0.81982	0
Yeosu (YS)	0.01156		0.08108	0.66667	0
Wando (WD)	-0.01022	0.04542		0.08108	0
Buan (BA)	0.01236	0.00056	0.05000		0
Jeju Island (JJ)	**0.75812***	**0.74826***	**0.76083***	**0.76325***	
Shanghai (SH)	0.15330	0.12592	0.15488	0.16125	**0**

Significant *P* values are indicated by * $P < 0.001$

Table 7. Distribution of mtDNA 16s rRNA haplotypes in six populations of *Mugil cephalus*.

Haplotype	Sampling site							n	%
	GS	BS	YS	WD	BA	JJ	SH		
H1	28	26	25	29	28	7	7	150	76.53
H2	1							1	0.51
H3		1						1	0.51
H4		1						1	0.51
H5		1						1	0.51
H6			1					1	0.51
H7				1				1	0.51
H8				1				1	0.51
H9					1			1	0.51
H10					1			1	0.51
H11					1			1	0.51
H12						30		30	15.31
H13						2	1	3	1.53
H14						2		2	1.02
H15						1		1	0.51
Total	29	29	26	31	31	42	8	196	100

Table 8. Summary of molecular diversity for *Mugilcephalus* in mtDNA 16s rRNA. Number of individuals (n), number of haplotype (*N*), haplotype diversity (*h*), nucleotide diversity (π), mean number of pairwise differences (*k*) for each population of samples. Tajima's D and Fu's Fs, corresponding P value, and mismatch distribution parameter estimates for each population were also indicated.

						Tajima's *D*		Fu's *Fs*		Mismatch distribution		
Region	**n**	**N**	***h***	**π**	***k***	***D***	***P***	***Fs***	***P***	**τ**	**θ0**	**θ1**
Goseong (GS)	29	2	0.0690 ± 0.0632	0.0001 ± 0.0003	0.0690 ± 0.1442	-1.149	0.133	-1.183	0.057	3.000	0.000	0.077
Busan (BS)	29	4	0.1995 ± 0.0977	0.0004 ± 0.0005	0.2069 ± 0.2606	-1.733	0.009	-3.324	0.000	3.000	0.000	0.262
Yeosu (YS)	26	2	0.0769 ± 0.0697	0.0001 ± 0.0003	0.0769 ± 0.1532	-1.156	0.141	-1.094	0.059	3.000	0.000	0.087
Wando (WD)	31	3	0.1269 ± 0.0798	0.0002 ± 0.0004	0.1290 ± 0.2007	-1.506	0.020	-2.397	0.006	3.000	0.000	0.152
Buan (BA)	31	4	0.1871 ± 0.0927	0.0004 ± 0.0005	0.1935 ± 0.2506	-1.731	0.009	-3.436	0.000	3.000	0.000	0.242
Jeju Island (JJ)	39	5	0.4681 ± 0.0848	0.0049 ± 0.0029	2.6144 ± 1.4264	0.369	0.670	2.909	0.906	0.000	0.000	99999.0
Shanghai (SH)	8	2	0.2500 ± 0.1802	0.0037 ± 0.0027	2.0000 ± 1.2562	-1.701	0.013	3.555	0.944	3.000	0.000	0.183

Table 9. Pairwise estimates of *F*st (below the diagonal) and Pairwise *F*st *P* values populations of *Mugil cephalus* in mtDNA 16s rRNA.

Locality	GS	BS	YS	WD	BA
Goseong (GS)		0.99099	0.79279	0.89189	0.91892
Busan (BS)	-0.00000		0.87387	0.45946	0.55856
Yeosu (YS)	0.00022	-0.00181		0.83784	0.88288
Wando (WD)	-0.00068	0.00053	-0.00156		0.99099
Buan (BA)	-0.00706	0.00008	-0.00263	-0.00000	
Jeju Island (JJ)	**0.77091***	**0.76278***	**0.76281***	**0.77294***	**0.76991***
Shanghai (SH)	0.16492	0.11689	0.14709	0.15575	0.13846

Significant *P* values are indicatd by * $P < 0.001$

ii. Genetic structure and phylogenetic relationships

(a) MtDNA COI

A minimum spanning network (MSN) of COI haplotypes showed two clearly distinctive clades. Clade 1 comprised haplotypes from all populations (H1~H11, H 18), and clade 2 comprised haplotypes from JJ and SH populations (H12~H17). Within clade 1, H1 was the most abundant haplotype and comprised a star like phylogenetic network containing 11 haplotypes (H2~H11, H18). In the network constructed with 6 haplotypes (H12~H17) from clade 2, H12 was the most abundant, but H13, sharing JJ population and 1 specimens in SH population, was connected to 5 other haplotypes by one or two steps (Fig. 4a).

The phylogenetic trees using neighbor joining (NJ) are shown that *Mugil cephalus* around Korea were divided into two major groups (Fig. 5a). Group 1 includes 160 individuals from all populations, and group 2 comprises 36 individuals from JJ and SH populations. Compared to mtDNA COI sequences of *Mugil cephalus* in Taiwan, group 1 including 12 haplotypes (H1~H11, H18) contained *Mugil cephalus* (JQ060540), recognized as lineage 1. And group 2 including 6 haplotypes (H12~H17) belong to *Mugil cephalus* (JQ060553) of

lineage 2. But, *Mugil cephalus* (JQ060541) in lineage 3 didn't closely cluster to any populations (Fig. 6). The genetic distance within group 1and group 2 was *d*= 0.000–0.005, respectively, but, the genetic distance between two groups was *d*= 0.021–0.029.

The results of Bayesian analysis showed two major groups that were consistent with the phylogenetic trees; group 1 and group 2 were confirmed as lineage 1 and lineage 2, respectively. This result was supported by high posterior probabilities (Fig. 7).

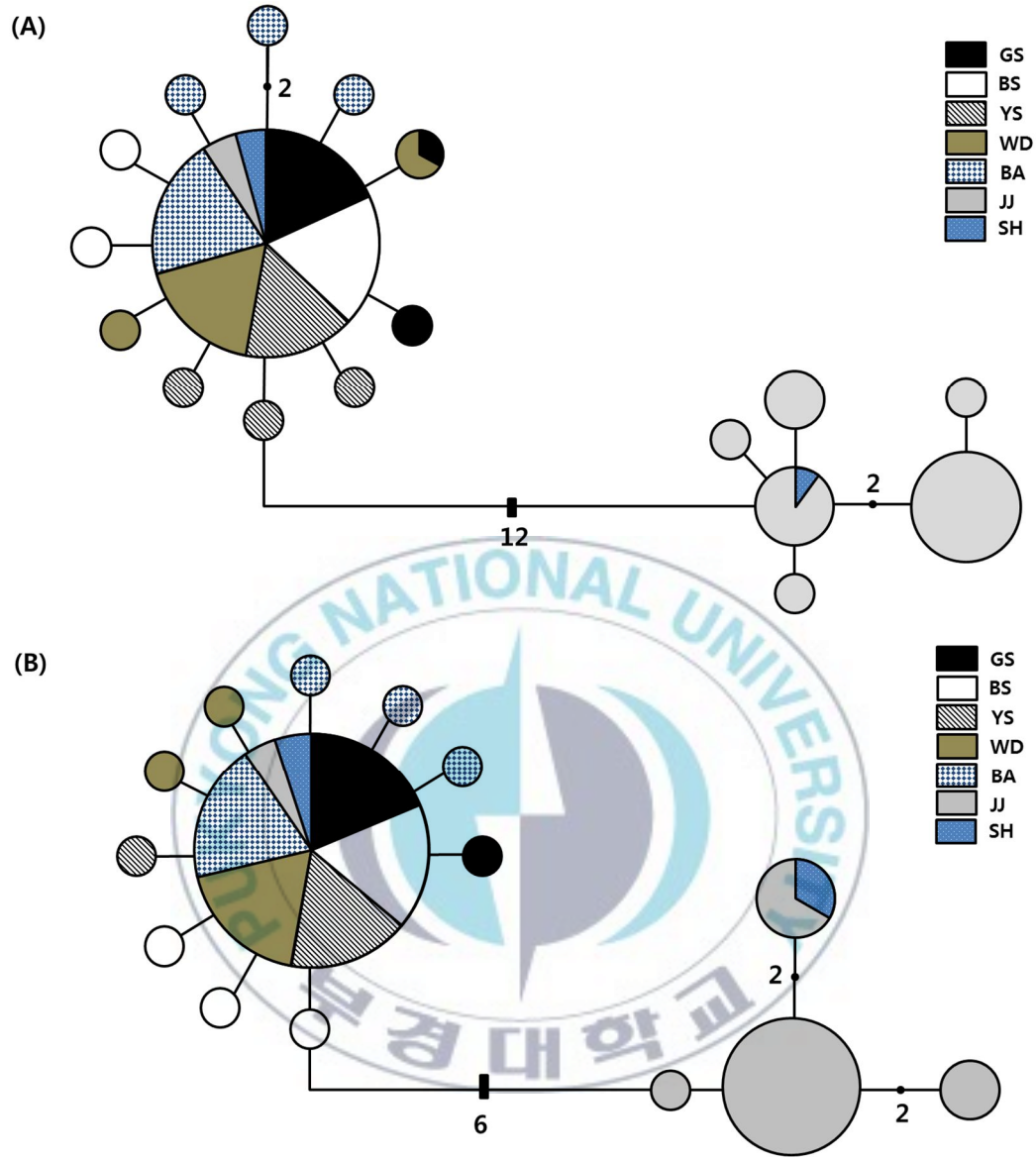

Fig. 4. Minimum spanning trees showing a genetic relationship among (A) COI gene haplotypes and (B) 16s rRNA gene haplotypes for three groups. The sizes of the circles are proportional to haplotype frequency. Thick marks on the lines joining haplotypes represent the number of nucleotide substitutions. GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

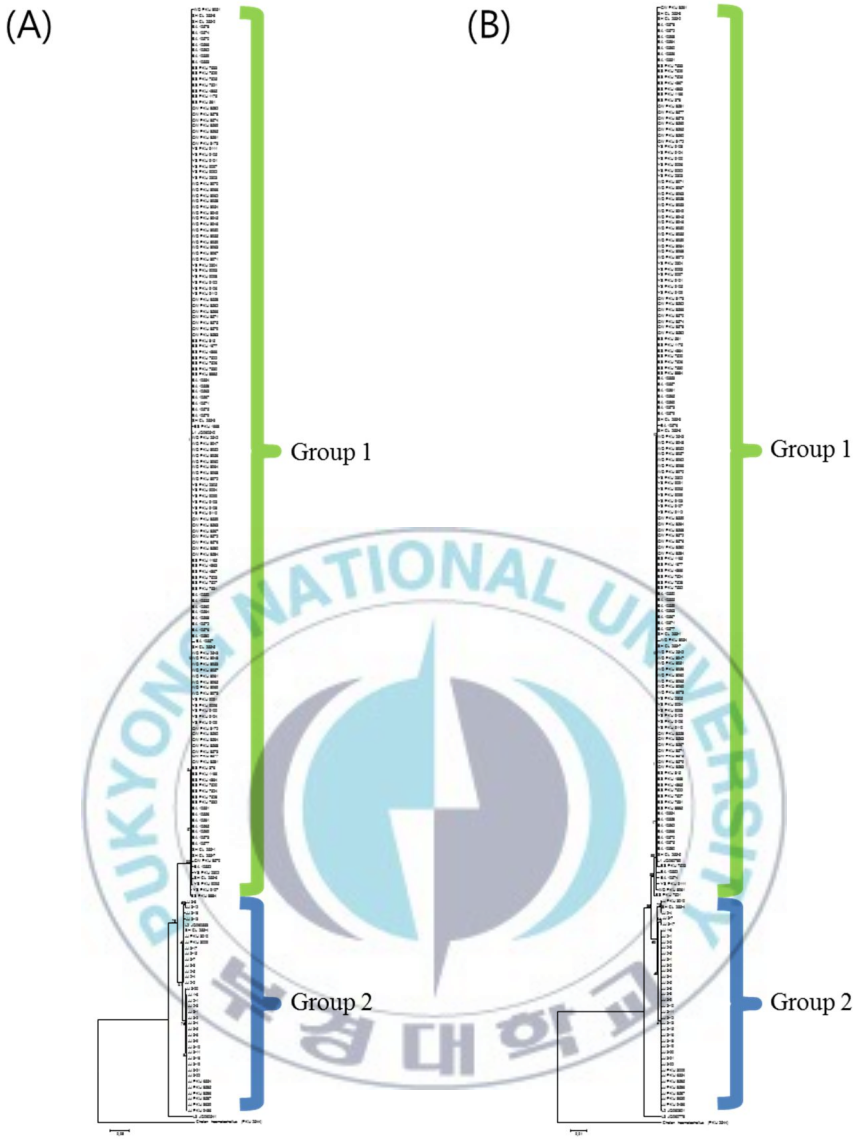

Fig. 5. Neighbor-joining tree showing the relationships among populations of *Mugil cephalus* using (A) mtDNA COI in 572 bp, and (B) mtDNA 16s rRNA in 541 bp. The NJ tree was constructed under the K2P model using *Chelon haematocheilus* as the outgroup. Numbers of branches indicate bootstrap probabilities in 1,000 bootstrap replications.

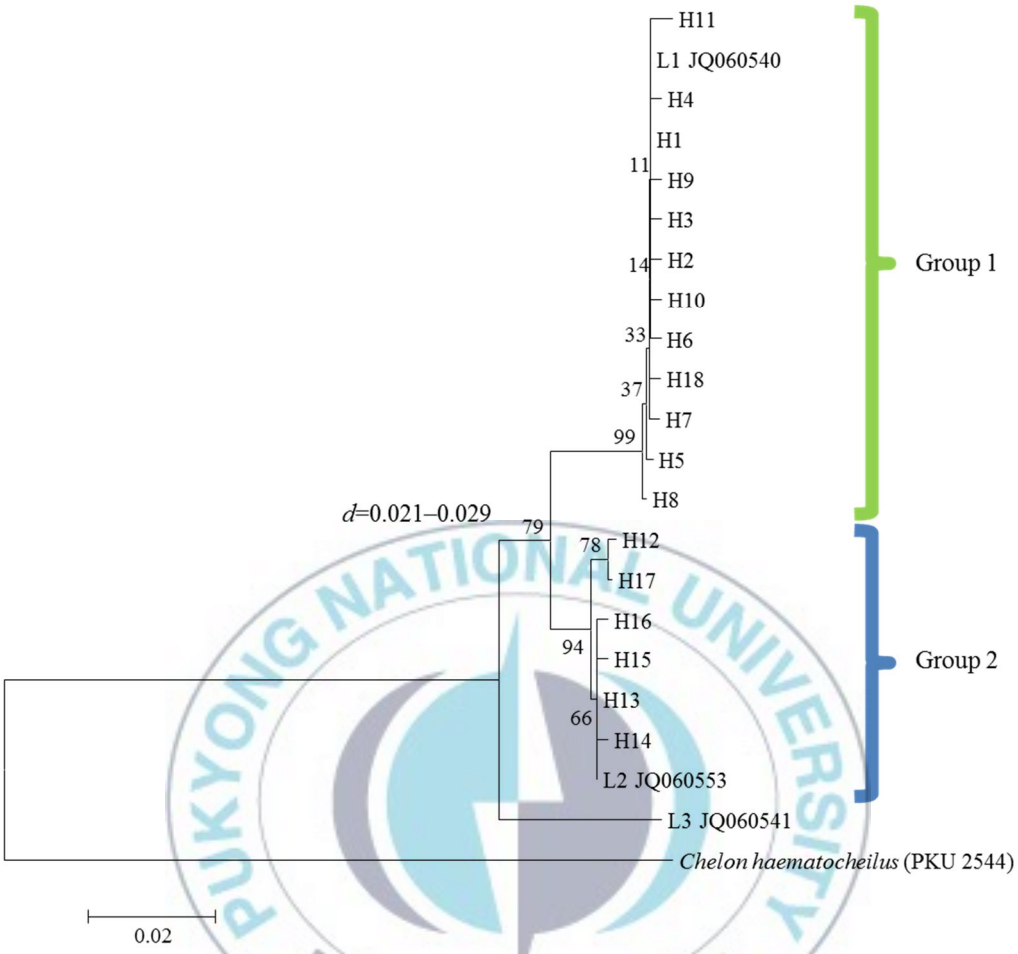

Fig. 6. Neighbor-joining tree for mtDNA COI haplotypes of *Mugil cephalus*. Bootstrap support in 1,000 replicates.

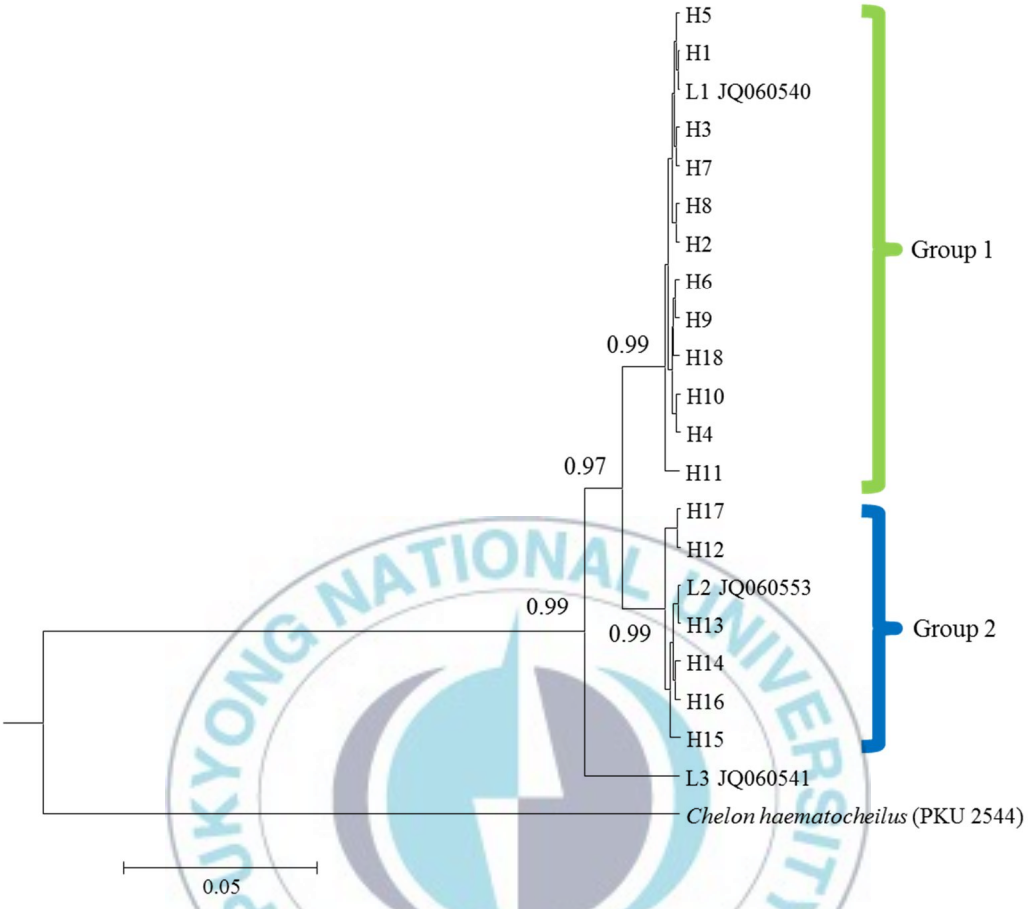

Fig. 7. Bayesian analysis of mtDNA COI haplotypes for *Mugil cephalus*. The phylogenetic tree was constructed under HKY+I model. Numbers of branches correspond to posterior probabilities.

(b) MtDNA 16s rRNA

In mtDNA 16s rRNA, the MSN identified the two clades for *Mugil cephalus*. Similar to COI results, clade 1 comprised haplotypes from all populations, and clade 2 comprised haplotypes from only JJ and SH populations. Clade 1 showed a so-called 'star'phylogeny pattern, with the central high frequency haplotype (H1) separated by one or more base differences from all populations, and connected other 10 haplotypes. Clade 2, mainly linked to the most abundant haplotypes (H12), was connected the other 3 haplotypes (Fig. 4b).

The phylogenetic tree obtained by the NJ method emphasized two separate groups of *Mugil cephalus* (Fig. 5b). Group 1 consisted of all populations and group 2 belongs to JJ and SH populations. The results of sequence comparison for *Mugil cephalus* in Taiwan showed that *Mugil cephalus* (JQ060789) is under group 1, and group 2 contained *Mugil cephalus* (JQ060801), designated as lineage 2 (Fig. 8). The genetic distances ranged d = 0.000–0.004, and d = 0.000–0.004 within group 1 and group 2, respectively, whereas two groups showed genetic differences from each other ranging from 0.010 to 0.012.

The Bayesian analysis carried out high posterior probabilities showed that *Mugil cephalus* in Korea were also divided into two groups; group 1 and group 2

were identified as lineage 1 and lineage 2, respectively (fig. 9).

Consistent with the phylogenetic analysis, therefore, the existence of two groups of *Mugil cephalus* in Korea is supported by NJ, Bayesian trees and MSN, and this result showed that all populations contained lineage 1, but lineage 2 comprised only JJ populatios in Korea (fig. 10).

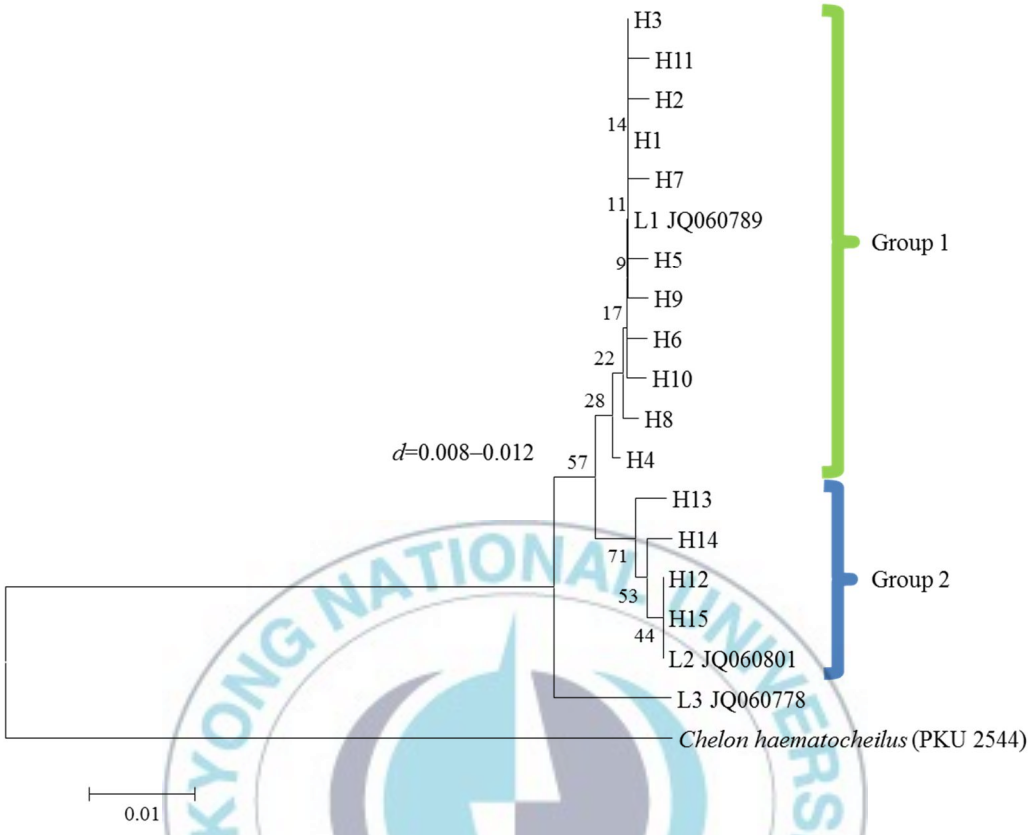

Fig. 8. Neighbor-joining tree for mtDNA 16s rRNA haplotypes of *Mugil cephalus*. Bootstrap support in 1,000 replicates.

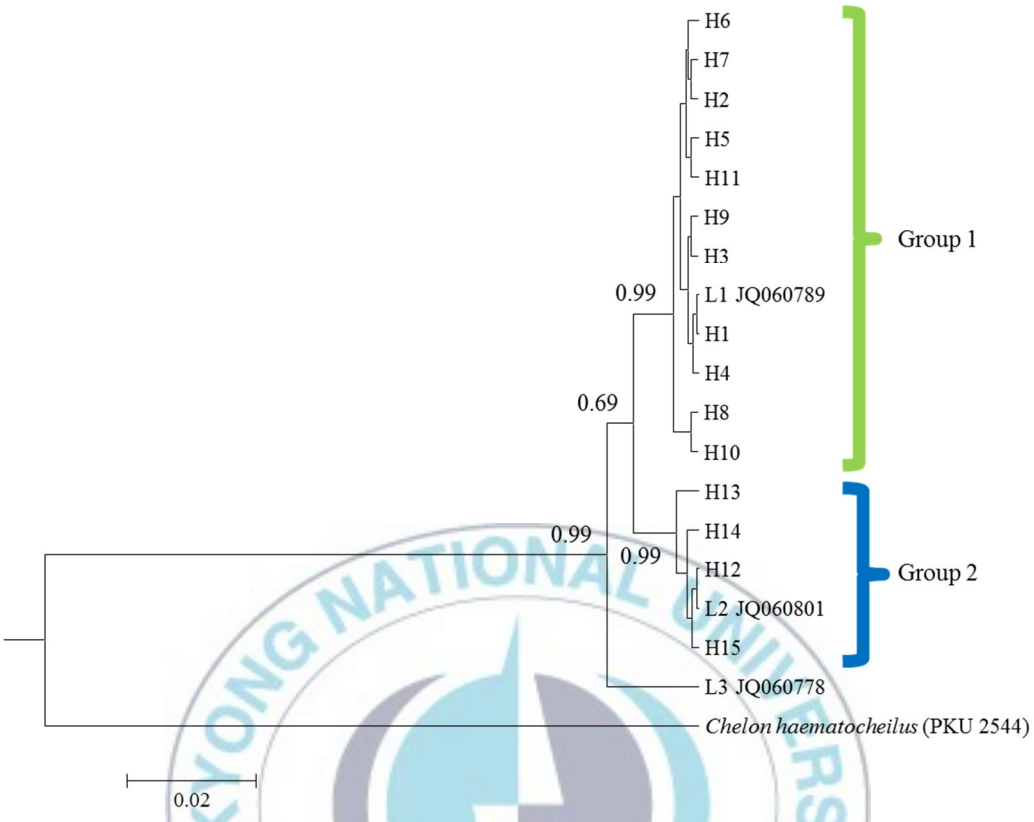

Fig. 9. Bayesian analysis of mtDNA 16s rRNA haplotypes for *Mugil cephalus*. The phylogenetic tree was constructed under HKY+I model. Numbers of branches correspond to posterior probabilities.

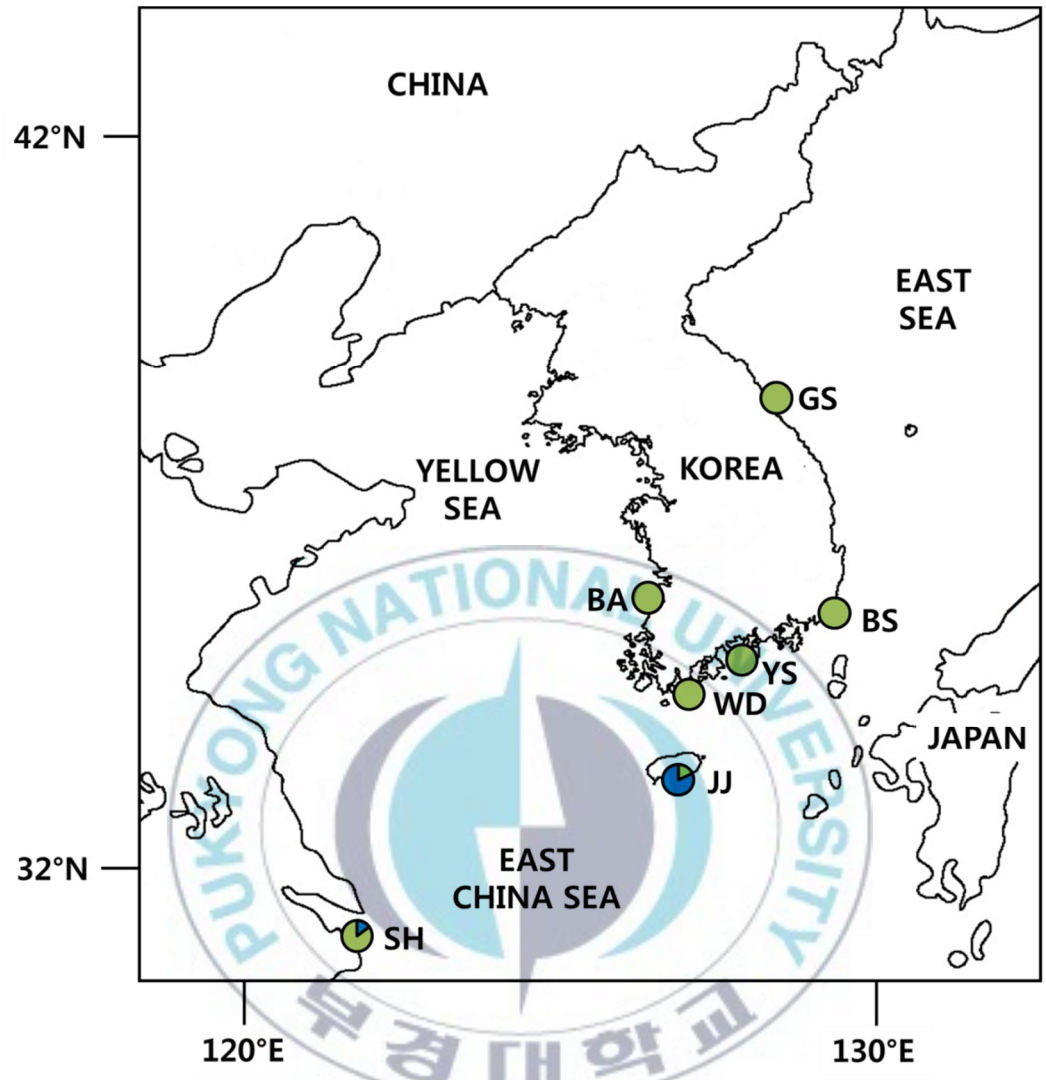

Fig. 10. The distribution of *Mugil cephalus*. The proportions of the lineage in six locations. Green: lineage 1, Blue: lineage 2. GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

iii. Demographic history of *Mugilcephalus*

(a) MtDNA COI

The tau value (τ), which provides a rough estimate of the time when rapid population expansion started, was equal values (3.000) in all populations except for JJ and SH. Also, in case of JJ population showing the lowestτ value, there is a great difference between before the expansion (θ_0) and after expansion (θ_1) (Table 5).

The mismatch distribution was unimodal for 5 populations (GS, BS, VS, WD, BA), whereas bimodal in JJ and SH populations (Fig. 11). To obtain more precise estimates, the neutrality analysis was performed, and Tajima's D and Fu's Fs showed negative values in 6 populations except for JJ populations. But, in Tajima's D test, only BS, YS, BA, SH populations had significant values ($P < 0.05$), and GS, BS, YS, BA populations were statistically significant in Fu's Fs test ($P < 0.05$).

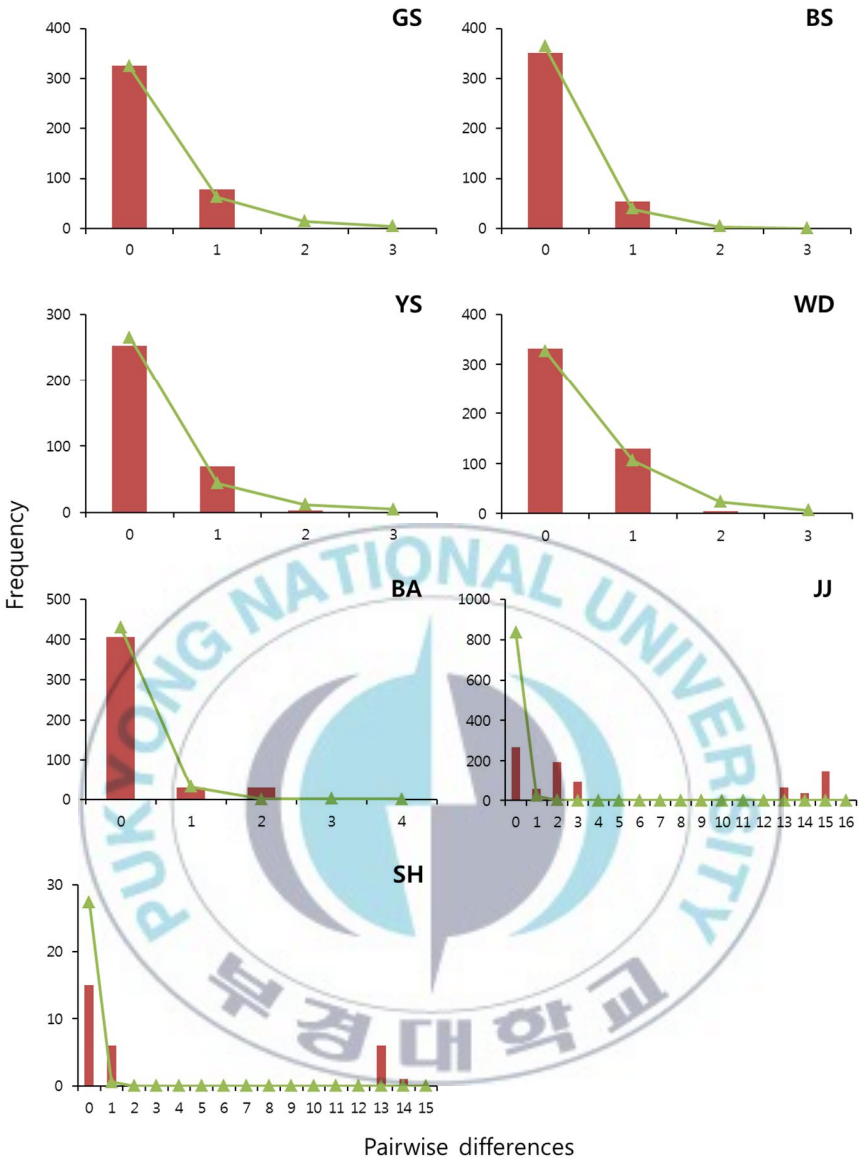

Fig. 11.Mismatch distributions from the mtDNA COI sequences of *M. cephalus* from seven sampling locations. Bar: observed distributions; Line: expected distributions from the sudden expansion model. GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

(b) MtDNA 16s rRNA

ARLEQUIN calculated the value of τ as 3.000 in all populations except for JJ population, and after expansion value (θ_1) was the highest in JJ population (99999.0), whereas GS population had the lowest value (0.077) (Table 8).

Mismatch distribution for 5 populations (GS, BS, YS, WD, BA) appeared to be unimodal, but the mismatch distribution for JJ and SH populations was bimodal (Fig. 12). The results of the two statistical tests, Tajima's D test and Fu's Fs test, showed that although all population except for JJ population was negative, Tajima's D test produced a statistically significant value in BS, WD, BA, SH populations ($p < 0.05$). Also, in Fu's Fs test, BS, WD, BA populations showed statistically significant values ($p < 0.05$).

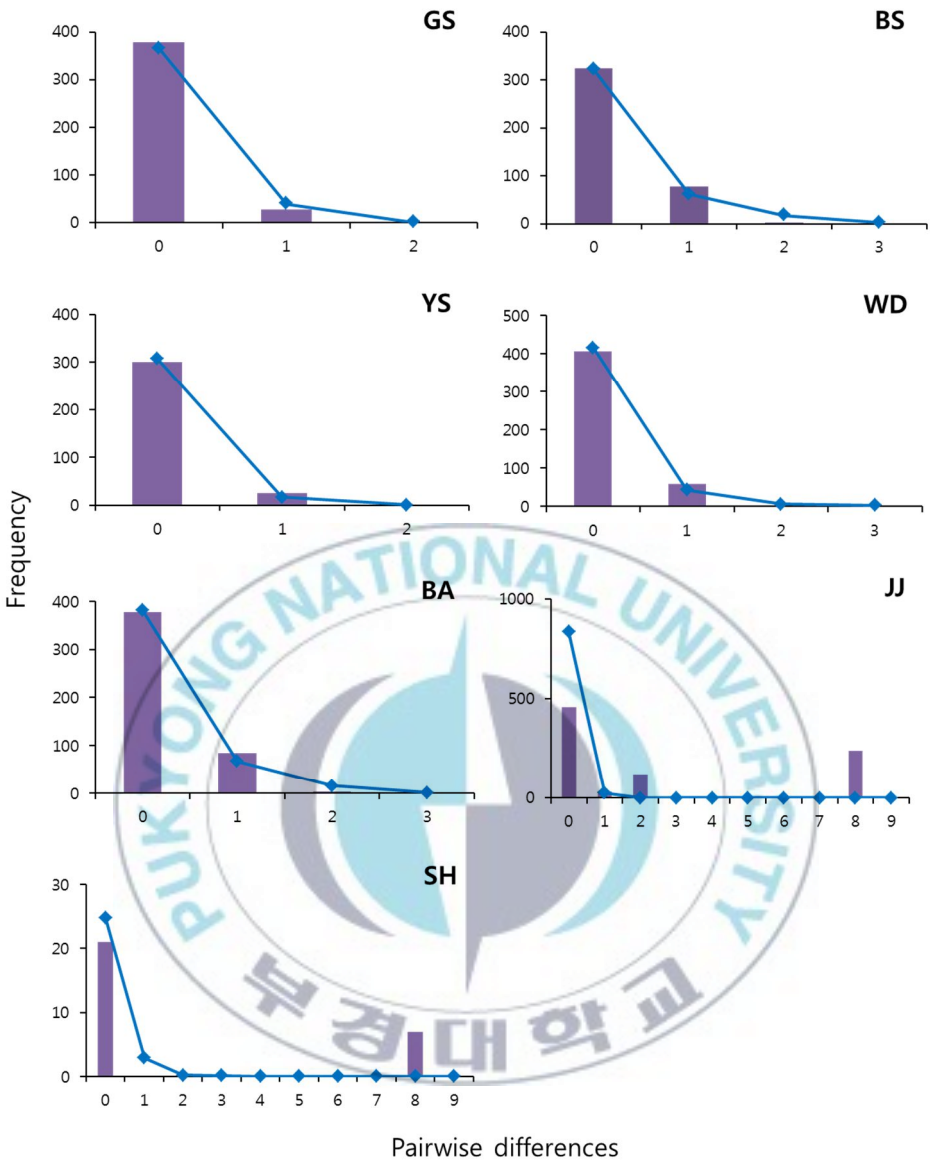

Fig. 12. Mismatch distributions from the mtDNA 16s rRNA sequences of *M. cephalus* from seven sampling locations. Bar: observed distributions; Line: expected distributions from the sudden expansion model. GS, Goseong; BS, Busan; YS, Yeosu; WD, Wando; BA, Buan; JJ, Jeju Island; SH, Shanghai.

iv. Divergence time

Generally, given that the divergence rate in the mtDNA COI sequences of marine fishes was approximately 2% per MY, according the Brown et al. (1982), the two clades in this study might have diverged at 1.15–1.4 MY. Because the divergence rate for mtDNA 16s rRNA of 1% per MY was known by Ni et al. (2014), the divergence time between two clades was estimated to be 1.0 to 1.2MY. So, two clades in *Mugil cepahlus* might have diverged at 1–1.2MY, indicating isolation in the late Pleistocene.

IV. Discussion

Genus *Mugil* comprises monophyletic clade, and among them, *Mugil cephalus* was divided into 14 lineages based on geographical distribution (Durand et al., 2012). Especially, three lineages coexisted in Taiwan, and there exist two lineages in China and Japan (Ke et al., 2009; Durand et al., 2012; Sun et al., 2012; Shen et al., 2011). According to Shen et al. (2011), *Mugil cephalus*, distributed in East China Sea including Korea, included lineage 1. However, this study showed that *Mugil cephalus* in Korea appeared to both lineage 1 and lineage 2, and the pairwise *F*st values showed significant differentiation between two lineages by 0.9717 and 0.9736 in COI and 16s rRNA, respectively. Especially, JJ and SH populations have both two lineages unlike other populations belonging to lineage 1. Also, genetic divergence between JJ and other populations estimated by pairwise *F*st was greatly significant ($P < 0.001$), indicating a clear differentiation among populations. Generally, such a great intraspecific genetic differentiation reflected the very restricted gene flow resulting from the existence of oceanic or terrestrial barriers (Graves, 1998; Rocha-Olivares et al., 2000). Therefore, it is estimated that barriersexist between lineage 1 and lineage 2.

1. What is the factor forming lineages of *Mugil cephalus* ?

i. Demographic history

There are no haplotypes sharing between two lineages (lineage 1 and lineage 2) in both mtDNA COI and 16s rRNA sequences, and this indicated that new, local mutations have accumulated in these two lineages with very little or no subsequent gene exchange (Salgueiro et al., 2004; Timmers et al., 2012). Also, it is evidence of their long genetic isolation. Compared to genetic diversity, lineage 2 has higher value than other populations in nucleotide diversity and haplotype diversity. The mtDNA COI genetic diversity of *Mugil cephalus* in northwestern Pacific showed that lineage 1 was low h (0.1316 ± 0.0295) and low π (0.0002 ± 0.0004), but lineage 2 was high h (0.6525 ± 0.0264) and low π (0.0021 ± 0.0015) (Shen et al., 2011). This corresponded with our result in mtDNA COI.

According to Grant and Bowen (1998), among 4 types of relationship between h and π, lineage 2 belongs to the second type, high h (0.5937 ± 0.0697) and low π (0.0022 ± 0.0016) of mtDNA COI, suggesting that this population is under rapid population expansion after a period of low effective population size and rapid

population growth enhances the retention of new mutations. Also, lineage 2 had bimodal in mismatch distribution, indicating historically differentiated allopatric populations or a somewhat restricted expanded species (Rocha-Olivares et al., 2000; Fauvelot et al., 2003; Ely et al., 2005; Kim et al., 2006). On the other hand, in lineage 1, L-shaped mismatch distribution with a zero peak reflected the single dominant haplotype with 1-2 mutational steps away. Also, low *h* (0.1894 ± 0.0419) and low π (0.0003 ± 0.0005) were closely fitted to a model of sudden demographic expansion (Harpending, 1994;Grant and Bowen, 1998;Salgueiro et al., 2004; Semina et al., 2007; Liu et al., 2007; Laakkonen et al., 2013).

Two lineages of *Mugil cephalus* in this study might have diverged at 1.0–1.4MY, indicating isolation in the late Pleistocene, which might occurred between approximately 0.01 and 1.8 MY (Liu et al., 2007; Shen et al., 2011). During the late Pleistocene, low sea levels resulted in the geographical isolation of species, and such conditions might derive a barrier between two lineages. Lineage 1 was isolated in the East Sea and lineage 2 in the East China Sea, which influenced strong genetic differentiation between lineage 1 and lineage 2 (Liu et al., 2006; Liu et al., 2007; Shen et al., 2011). Glacial period played an important role in making current genetic diversity patterns in many marine organisms (Kokita &

Nohara, 2012). Glacial survivor populations were potentially subject to geographic isolation with genetic drift restricted to several separated glacial refugia (Hu et al., 2011). For example, during Pleistocene low sea levels, *Chelon haematocheilus* in Northwestern Pacific were divided into three lineages, which might have diverged in the three marginal seas (East Sea, East China Sea and South China Sea), and three distinct lineages of *Lateolabrax japonicus* were also detected (Liu et al, 2006; Liu et al., 2007).

During the period, fluctuations in the sea level as well as temperature, salinity and ocean currents had a great effect on the demography and genetic diversity of fish species, and this led to phylogeographical structure (Hewitt, 2000; Jamandre et al., 2009; Kokita & Nohara, 2012; Pauls et al., 2013). From the Pliocene to the last glacial maximum, the basin of the East China Sea, the Yellow Sea and the southern coastal region of Korea were exposed, and the southern coast of Korea was also connected to Jeju Island (Liu et al., 2006; Song et al., 2010; Lee et al., 2012). However, as sea levels rose after the LGM, the coastline migrated landward from Jeju Island in Korea to the Bohai Gulf in China (Xu and Oda, 1999; Yang et al., 2009). These sea level changes might have inevitably influenced the spatial distribution and genetic patterns of marine species inhabiting the region

(Ni et al., 2014). Thus, although two lineages were diverged by geographical isolation during the glacial period, free migration after the sea level rose might give an opportunity for two lineages to coexist in Jeju Island.

ii. Oceanic currents

Another factor, which has an effect on current distribution of each lineage for *Mugil cephalus*, is oceanic currents (Shen et al., 2011). Generally, because marine fishes have the high dispersal ability, larval transport by present-day currents is important in the contemporary gene flow (Ke et al., 2009; Shen et al., 2012; Kokita & Nohara, 2012).

Shen et al. (2011) suggested that distribution range of three lineages in *Mugil cephalus* was probably facilitated by the oceanographic currents. Lineage 2 appears to match the circulation pattern of the Kuroshio Current. Lineage 3 was distributed following the warm South China Current, whereas lineage 1 appears to be restricted to the cold North China Coastal Current. This distribution range of each lineage by oceanographic current systems might be associated with the temperature preferences. *Mugil cephalus* can migrate along coasts and between continental and open sea water environments during life cycle and have slightly different temperature preferences (Whitfield et al., 2012). The temperature differences between Queensland and New South Wales played a role in determining genetic differentiation of two lineages in *Mugil cephalus* (Kruck et al., 2013).

There are various ocean currents or water mass such as Tsushima Warm current, Kuroshio Current, East Korea Warm Current, Western Korea Coastal Water, Jeju Warm Current, and Yellow Sea Bottom Cold Water, Liman Current around Korea, and a variety of water temperature and salinity front played a barrier role in limiting distribution and migration of fishes (Yang et al., 1998; Kim et al., 2005; Lee et al., 2012). Especially, Jeju Island and South Sea showed the very complex oceanic condition, which mixed many different oceanographic currents such as Tsushima Warm Current, Jeju Warm Current, South Korean Coastal Water, Yellow Sea Bottom Cold Water, Yellow Sea Coastal Water, and Changjiang Diluted water (Chen et al., 2009; Bae and Kim, 2012; Choi et al., 2011).

According to Kim et al. (2005), Jeju Strait between southern sea and Jeju island has low salinity and temperature, whereas Korea Strait between Jeju and Kyushu Island showed high salinity and temperature, which was influenced by Tsushima Current. The Kuroshio Current played an important role in the driving mechanisms of the Tsushima Warm Current (TSWC) and Yellow Sea Warm Current (YSWC); the origin of the TSWC is considered to be the Kuroshio Branch Current west of Kyushu (KBCWK) and YSCW is considered to be the

current bifurcated from the KBCWK south of Jeju Island (Ichikawa & Beardsley, 2002; Guo, 2006). The Kuroshio and Tsushima Warm Current are dominant and exist in both winter and summer, but the Yellow Sea Warm Current exists only in winter (Ichikawa & Beardsley, 2002). Also, Lin et al., (2001) confirmed that the current south of Jeju Island is variable and exhibits significant eddy motion in summer, and existence of an annual mean Jeju Warm Current in winter. Therefore, Jeju Island might be directly influenced by warm currents both winter and summer. On the other hand, various sources of low temperature such as the North Korea Cold Water in East Sea, South Korean Coastal Water, and the expansion of the Yellow Sea Bottom Cold Water and South Sea Bottom Cold Water in summer could have an effect of the distribution temperature of lineage 1 (Cho and Kim, 1994; Choi et al., 2011).

According to Kim et al. (2005), the movement of *Mugil cephalus* trended toward an inner bay and north bound mainly. So, *Mugil cephalus* in South Sea showed a tendency to migrate along the inner bay, and this coincided with the distribution of lineage 1 except for Jeju Island. Therefore, lineage 1 was adapted to low temperature whereas lineage 2 preferred to live high temperature, and ecological differences between two lineages might play a role as a barrier.

In conclusion, *Mugil cephalus* in Korea was divided into two distinct groups according to the geographic distribution pattern, and the phylogeographical structure and demographic histories of two groups might be influenced by the result of postglacial colonization,and the two groups may be maintained by the present oceanographic condition.

2. Different species?

The genetic distances between lineage 1 and lineage 2 were 2.1–2.9% in mtDNA COI and 1.0–1.2% in 16s rRNA. Also, this study obtained a very significant differentiation value between two lineages, indicating that they are genetically distinct ($P < 0.001$). However, it is difficult to determine how extent the genetic differences found between *M. cephalus* populations may reflect their phenotypic differences (Semina et al., 2007). For a long time, morphological features such as meristic and morphometric characters were used to identify marine fish populations (Ibañex et al., 2007; Jorgensen et al., 2008; Gonzalez-Castro et al., 2012). However, because mugilid fishes are very similar in external shape, they are taxonomically confusing groups at intraspecific or interspecific levels (Rocha-Olivares et al., 2000; Semina et al., 2007; Menezes et al., 2010).

Although many morphological characters were proposed by many authors to reveal taxonomic relationships of mugilid fishes, there are few characters to clearly establish the relationships among mugild fishes (Caldara et al., 1996; Nirchio et al., 2005; Heras et al., 2009; Ashiq Ur Rahman et al., 2013). So, this confusing taxonomy in mugilidae was given by the large synonymy, and although

mugilidae includes up to 233 nominal species, only 80 of them have been recognized as a valid species (Nirchio et al., 2005; Shan-Hu et al., 2011; Froese & Pauly, 2012; Siccha-Ramirez et al., 2014).

In our study, the results of expected affiliated groups showed that two lineages could be classified correctly with an accuracy of 98.4%, indicating that two lineages are morphologically distinguished. However, in meristic characters, six Korean populations didn't show differences, but adult and juvenile displayed a significant difference in the number of anal fin spines and soft rays. According to Wallace and Elst (1975), when juvenile reached to 55mm in standard length, the number of anal spine and soft rays changed 2 spines and 9 rays (II, 9) to 3 spines and 8 rays (III, 8). The juveniles in this study were 22.94–38.01 mm in standard length, and this indicated the pre-metamorphosis stage of the last anal fin spines. In the case of morphometric characters, on the other hand, JJ population showed the higher values in head length, body depth and caudal peduncle depth than the other populations. According to Kim (1999), *Mugil cephalus* of Jeju Island has higher body depth and wider interorbital width than the other area populations. However, in our CDA analysis, JJ population was distinct from other populations in caudal peduncle depth.

Generally, morphological traits were influenced by environmental as well as genetic variation (Jorgensen et al., 2008; El-Zaeem, 2011). Baltic Sea herring (*Clupea haregus*) have been shown to exhibit morphological differences in skull shape across the marked salinity and temperature gradients in the region (Jorgensen et al., 2008), and in case of *Leucopsarion petersii*, genetically divided into two lineages, East Sea population tend to have larger body size and many more vertebrae than those of the Pacific population (Kokita and Nohara, 2011). *Maurolicus muelleri*, which was considered as a synonym of *Maurolicus japonicus*, was laterrecognized as a valid species because of morphometric and molecular differences depending on the geographical distant distribution (Habib et al., 2012).

Mugil cephalus is widely distributed in very various environments such as coastal waters and estuaries of the tropical and temperate waters in the world (Nelson, 2006; El-Zaeem, 2011; Kwun et al., 2013). Corti & Crosetti (1996) suggested that the meristic character of *Mugil cephalus* was partially associated with the geographic origin. In case of *Mugil cephalus* in Mexico, population in Atlantic coast has wider body width, but another population in Pacific coast was the narrower body (Ibáñez-Aguirre et al., 2006). Also, Heras et al. (2006)

suggested that *Mugil cephalus*, which distributed in South America, were identified as *Mugil curema* through the morphological and molecular analysis. And *Mugil* sp. from western north Atlantic showed very significant statistical differences from *Mugil cephalus* in number of transverse scale rows, horizontal scale rows and circumpeduncular scale rows, accordingly it is revealed that these individuals might be a population of *M. liza* (Menezeds et al., 2010). Therefore, the environmental difference was associated with morphological diversity.

This study revealed that there are morphologically and genetically two distinct lineages in Korea. Especially, JJ population, which both lineages coexisted, is distinguished from other populations in molecular and morphological traits. Similarly, three lineages in Taiwan formed genetically distinct clusters in the results of microsatellite as well as mitochondrial genetic markers. Therefore, each lineage is reproductively isolated, and this reproductive isolation prevented further genetic exchange between populations and potentially leading to speciation (Ke et al., 2009; Alcázar et al., 2012; Shen et al., 2012; Kokita & Nohara, 2012).

Compared to spawning period of Jeju and Yeosu populations, both lineages are November to January (Kim et al., 2004; Zhang et al., 2011). Some studies revealed that *Mugil cephalus* exhibits variation in reproductive strategies; some

population return to estuaries following spawning, and others may remain within the marine environment (Ke et al., 2009; Shen et al., 2011; Whitfield et al., 2012). So, it might be that lineage 2 is the group setting within the marine environment. To reveal this problem, it is required to study the exact spawning ground and period of two lineages.

Chyung (1997) suggested that *Mugil cephalus* in Jeju Island have higher body depth unlike other areas, and named this population as *Mugil japonicus*. Also, according to Kim (1999), it needs to reveal whether *Mugil japonicus* is a valid species because *Mugil cephalus* in Jeju Island shows higher body depth. *Mugil japonicus* is currently treated as the synonym of *Mugil cephalus* (Lee and Joo, 1994; Whitfield et al., 2012; Kottelat, 2013). In order to clarifythe taxonomic status of JJ population in lineage 2 of *Mugil cephalus*, the further study is also required such as skeletal structure and microsatellite DNA.

V. References

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VI. Acknowledgement

부족한 제가 주위에서 도와주시는 많은 분들 덕분에 이렇게 2년을 잘 마무리할 수 있었습니다. 그 고마운 마음을 일일이 찾아 뵙고 감사드리는게 마땅하나 이렇게 짧은 글로 감사의 마음을 전하고자 합니다.

먼저 학문적으로나 인성적으로 한 단계 성장할 수 있도록 격려해주시고, 지도해주신 김진구 교수님께 깊은 감사의 말씀을 드립니다. 교수님께서 많은 기회를 얻을 수 있도록 항상 도와주시고 배려해주신 덕분에 제가 이렇게 성장할 수 있었습니다. 정말로 감사합니다. 좋은 논문으로 완성될 수 있도록 꼼꼼하게 심사해주시고 아낌없는 조언해주신 김현우 교수님, 유정화박사님 감사드립니다. 학부과정부터 많은 가르침을 주신 남기완 교수님, 김수암 교수님, 오철웅 교수님, 백혜자 교수님, 박원규 교수님께도 깊은 감사의 말씀 올립니다.

많은 분들의 도움 덕분에 이 논문을 완성할 수 있었습니다. 강원도 숭어샘플링에 도움주신 박정호 선배님, 여수·완도 샘플링에 함께해준 이우준오빠, 제주도 샘플링에 힘이 된 장인철군, 명세훈오빠, 마지막으로 힘들었던 서해안 숭어 샘플링에 큰 도움주신 국립수산과학원 서해수산연구소 박지영 언니, 함께 서해가서 고생했던 송영선양, 유효재 오빠께도 감사를 전합니다.

2년 동안 어류학 실험실에 있으면서 정말로 행복했습니다. 항상 웃는 얼굴로 맞이해주시고 따뜻한 조언을 아끼지 않으신 박경동 박사님, 반태우 선배님, 박정호 선배님께 감사의 말씀을 드립니다. 큰오빠처럼 옆에서 항상 힘이 되어주시고, 가끔 따끔하게 충고도 해주시는 수정이 오빠, 어려운 문제가 생기면 해결사처럼 도와주는 혁준이오빠, 묵묵히 옆에서 지켜보다가 한 마디씩 조언해주시는 환성이오빠, 정말로 감사드려요. 대학원와서 더 친해진 내친구 영선

이, 니가 있어서 진짜 든든했고, 말로 표현할 수 없을 정도로 고맙다. 매번 귀찮게하지만 짜증내지않고 누구보다 열심히 도와주는 동기 세훈이오빠, 우리 방 분위기 메이커 효재오빠, 자신의 분야에서 열정적인 우준이오빠, 4차원 상윤이오빠, 항상 도움 받기만 하네요, 감사합니다. 친언니처럼 챙겨주시고 함께 있으면 즐거운 권내림언니, 박꽃님 언니, 언니들 덕분에 즐거웠습니다. 동생이지만 오빠 같은 우리 인철이, 무한 매력녀 재민이, 우리방 이쁜 막내 서하랑 혜지, 이제 막 들어온 의철이, 모두 열심히 하니깐 잘할거라 믿고 옆에서 항상 응원할께. 고맙다. 또한, 각자의 전공 분야에서 원하는 목표를 위해 열심히하고 좋은 결실을 맺는 전유진양, 신아리양, 김효은양, 김정연양, 윤태호 오빠에게도 고마운 마음을 전합니다. 신입생 OT에서부터 지금까지 소중한 추억을 함께 한 최은혜, 허윤영, 안지혜, 김성희, 박다정, 옆에서 응원해줘서 고맙고 사랑합니다. 서로 멀리 떨어져 있지만 언제나 마음만은 함께인 친구들, 늘 저를 아껴주시는 모든 지인분들께도 감사의 말씀드립니다.

지금까지 옆에서 힘이 되어주고 묵묵히 지켜봐준 최고의 남자친구, 우정운님께도 감사합니다. 마지막으로 27년동안 언제나 저를 믿어주시고 뒷바라지 해주신 부모님, 정말로 감사드리고 사랑합니다. 세상 무엇과도 바꿀수 없는 소중한 배지은, 배주연양에게도 고마움을 전합니다.

되돌아보면 2년이라는 시간 동안 많이 배우고 과분한 사랑을 받았습니다. 그에 보답하도록 늘 발전하고 노력하는 사람이 되겠습니다. 감사합니다.